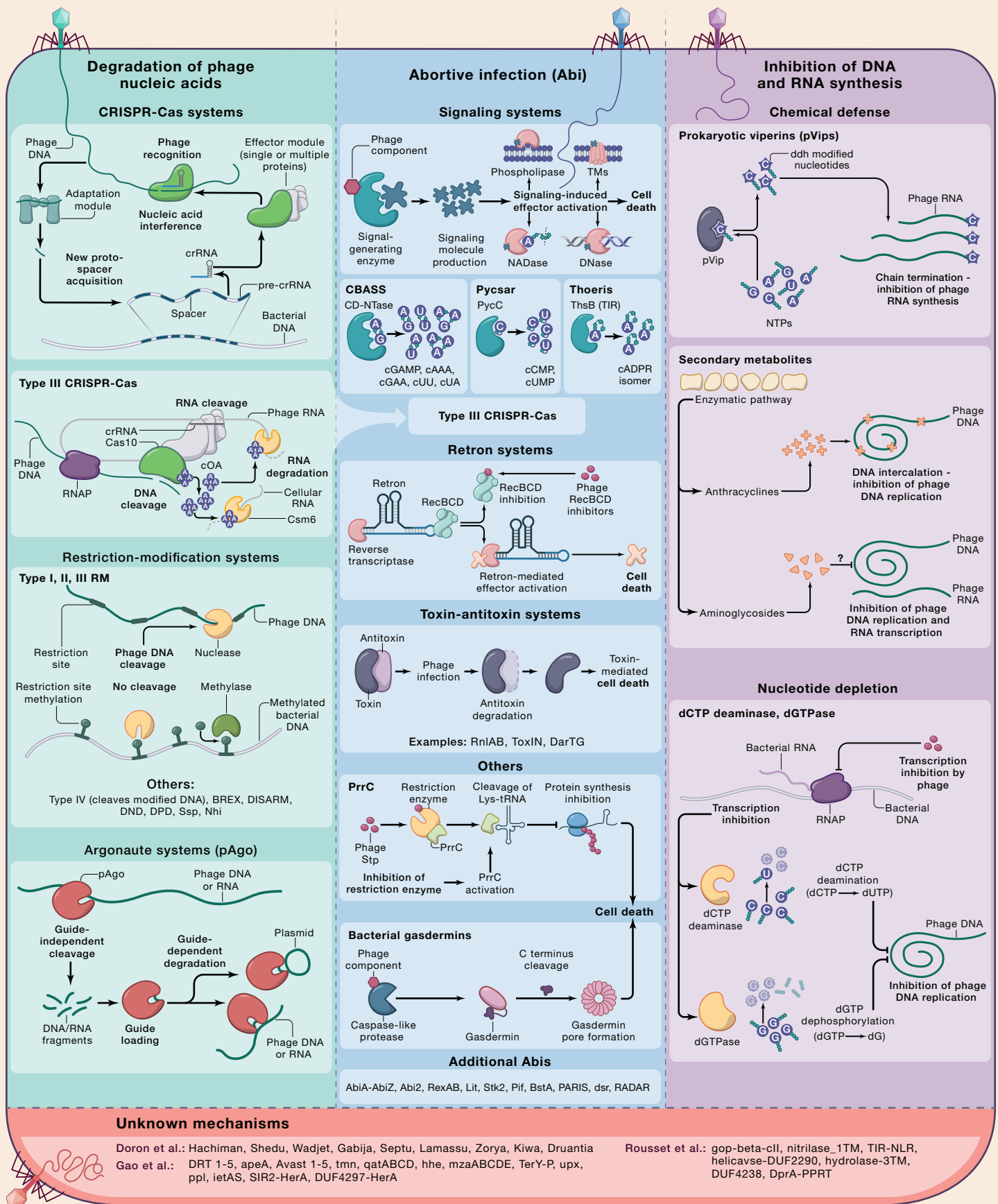


# SnapShot: Bacterial immunity

Cell

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Viral infection is often detrimental to bacterial cells, as most phages kill the infected cell at the end of the lytic infection cycle. Once attached to the cell surface, phages inject their nucleic acids into the host, where they are replicated, transcribed, and translated to produce new viral proteins and progeny phages. Bacterial defense strategies have evolved to target various stages of the infection cycle. Different bacterial strains encode different sets of anti-phage defense systems, and while no bacterium is known to encode all possible defense systems, for simplicity we present them visually as occurring within a single meta-cell.

## Degradation of phage nucleic acids

The most abundant defense systems in the microbial world are those that target and degrade phage nucleic acids. Restriction-modification (RM) systems cleave phage DNA upon recognition of specific sequence motifs. In most cases, these systems contain at least two components: one that recognizes the specific sequence in the bacterial genome and acts to modify it, in most cases by methylation on adenine or cytosine bases, and another that recognizes unmodified motifs in the DNA of the viral invader and acts to cleave it. Variations include type IV restriction enzymes that target modified phage DNA and non-methyl DNA modifications such as 7-deazaguanine and sulfur modification of the DNA backbone. CRISPR-Cas systems act through recognition and interference with phage nucleic acids through adaptive immune memory. The immune memory is obtained by acquiring short viral DNA sequences incorporated into the bacterial genome ("spacers"). These sequences are transcribed, processed, and loaded onto the CRISPR-Cas machinery, where they guide it to interfere with the viral DNA or RNA and prevent further infection. Type III CRISPR-Cas systems sense transcription from the phage genome and then, in addition to cleaving phage nucleic acid, generate cyclic oligoadenylate (cOA) signaling molecules that activate downstream effectors, which kill the infected cell or lead to growth arrest. Prokaryotic argonautes (pAgo) are also involved in nucleic acid-guided cleavage of phage DNA and RNA, with the aim of restricting viral propagation.

## Abortive infection

Forming a major strategy of bacterial defense against phages, abortive infection (Abi) systems act by killing the cell when they recognize phage infection. These systems kill the cell prior to the maturation of the phage progeny, preventing the spread of phages to neighboring cells and protecting the bacterial community. Abi systems have been described since the 1950s, and various such mechanisms are characterized. A common form of Abi includes signaling systems that, once they recognize phage infection, produce a signaling molecule that then activates a cell-killing effector. CBASS, Pycsar, and Thoeris are different types of signaling systems. In CBASS systems, enzymes of the CD-NTase family produce cyclic oligonucleotides as the signaling molecule, while Pycsar systems produce cyclic CMP (cCMP) or cyclic UMP (cUMP). Thoeris systems produce a signaling molecule that is an isomer of cyclic ADP-ribose (cADPR). In type III CRISPR-Cas, recognition of phage nucleic acids by the effector module triggers the production of a cOA signaling molecule (cOA) that activates an Abi response. Retron systems utilize a DNA-RNA hybrid (msDNA) that, together with a reverse transcriptase and effector proteins, guards cellular components. Inhibition of these cellular components by phage proteins activates the retron and leads to cell death. Several toxin-antitoxin (TA) systems were shown to become activated upon phage infection and lead to cell death or growth arrest. Other Abi systems include PrrC, a toxin that becomes activated when restriction enzymes are inhibited by phage proteins, and additional systems that inhibit cell growth by targeting tRNAs, forming membrane pores, phosphorylation of cellular proteins, inducing pre-mature cellular lysis, and more.

## Inhibition of DNA and RNA synthesis

In recent years, some anti-viral mechanisms were shown to directly inhibit phage DNA and RNA synthesis. Defense systems that act via chemical defense produce small molecules that poison the synthesis of phage nucleic acids. Prokaryotic viperins (pVips) were shown to produce several types of RNA chain terminator molecules. Anthracyclines inhibit phage infection, probably by intercalating into phage DNA and preventing DNA replication. In addition, aminoglycoside antibiotics were recently shown to inhibit viral replication via a yet unknown mechanism. An additional defense mechanism causing DNA replication inhibition is achieved by defensive enzymes that deplete deoxynucleotides, such as dCTP deaminase and dGTPase. These are triggered upon infection to eliminate one of the DNA nucleotides, consequently damaging the ability of the phage to replicate its genome.

## Defense systems with unknown mechanisms

Over the past few years, multiple studies have identified a large number of bacterial anti-phage defense systems whose mechanisms of defense remain unknown. These were identified based on their association with known defense systems in defense islands and were verified experimentally as providing defense against phages. Future studies are expected to shed more light on the mechanisms of these new systems.

## Abbreviations

RM, restriction modification; BREX, bacteriophage exclusion; DISARM, defense island system associated with restriction-modification; Dnd, DNA degradation phenotype; DPD, 7-deazapurine in DNA; CRISPR, clustered regularly interspaced short palindromic repeats; Cas, CRISPR associated proteins; pAgo, prokaryotic argonaute; CBASS, cyclic-oligonucleotide-based anti-phage signaling system; Pycsar, pyrimidine cyclase system for antiphage resistance; TA, toxin-antitoxin; pVip, prokaryotic viperin; TMs, transmembrane domain; cADPR, cyclic adenosine diphosphate ribose; CD-NTase, cGAS/DncV-like nucleotidyltransferase.

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## DECLARATION OF INTERESTS

R.S. is a scientific cofounder and advisor of BiomX and Ecophage.

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